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From the Research Laboratory of the Department of Health, New York City.

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THE RESULTS OF THE USE OF REFINED DIPHTHERIA ANTITOXIN, GIBSON'S "GLOBULIN PREPARATION," IN THE TREATMENT OF DIPHTHERIA.

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All who use diphtheria antitoxic serum extensively are aware that in from 10 to 30 per cent. of the injected cases of diphtheria, pronounced rashes, of an urticarial or erythematous type, occur. In the majority of cases these serum effects are disagreeable rather than harmful, but occasionally the rash is accompanied by constitutional disturbance presenting, in the most severe cases, high temperature, vomiting, prostration, and sometimes other symptoms. These marked constitutional reactions are especially likely to follow very large injections of from 10,000 to 20,000 units in young children who have high temperatures due to bronchopneumonia or other complications. In these cases the serum reaction is distinctly harmful, for by lowering the general resistance of the body to other infections it neutralizes to some extent the good done by the neutralization of the diphtheria toxin by the antitoxin. Furthermore, the rashes, especially those of a scarlatinal type, are puzzling in a diagnostic sense.

There have been many attempts made to separate diphtheria antitoxin from the non-antitoxic portions of the accompanying serum. Those interested in the chemical side of these investigations are referred to the recent article by Gibson.[1] In 1900 Atkinson, working in this laboratory, eliminated all but the globulins from the antitoxic serum. This partially refined antitoxic serum was tried in 36 cases. The results,[2] both as to antitoxic effect and

[1] Journal of Biological Chemistry, vol. i, Nos. 2 and 3.

[2] Archives of Pediatrics, November, 1900.

serum reactions, were so nearly identical with those in an equal number of cases treated with the whole serum from the same horse that it did not seem to be worth while to go to the expense of preparing such an antitoxic solution. Attempts to effect a practical separation of the antitoxin from a greater portion of the proteid non-antitoxic substances of the serum were continued. In August, 1905, we began trials with an antitoxic solution from which much more of the serum proteids had been eliminated than in the Atkinson preparation. Dr. R. B. Gibson, bacteriologist in the Research Laboratory, placed the half-saturation ammonium sulphate precipitate derived from the antitoxic serum in saturated sodium chloride solution, and found that along with a portion of the globulins all the antitoxin passed into solution. In this way the nucleoproteids and the insoluble globulins present in the Atkinson preparation were eliminated. The soluble globulins precipitated by acetic acid were filtered, partially dried, and finally placed in a sac of parchment membrane and dialyzed in running water. This antitoxic solution of soluble globulins was then rendered neutral, and sufficient sodium chloride was added to make it isotonic.

In carrying out the process there is a loss of about 30 per cent. of antitoxin units, because of retention upon filters, loss in dialyzing, etc. On testing this solution on a number of children we found that the results were favorable, except that more local pain was produced than with the whole serum. Stricter attention to the neutralization soon overcame this, so that when the serum was injected on one side and the globulin solution on the other the patient was unable to tell the one from the other. In October, 1905, the antitoxic globulin solution was administered by the medical inspectors not only in the hospitals for diphtheria but also in private homes. Since December it has been gradually distributed throughout New York City, and is now the only form of antitoxin supplied by the Health Department.

Results from the Use of Antitoxic Globulin Solution. The antitoxic effect was identical with that of the whole serum. Our tests have shown that not only the toxins and the so-called toxones produced in media by diphtheria bacilli, but also those produced in the animal by injections with living diphtheria bacilli are neutralized completely by the globulin solution. We could not detect the slightest evidence that any desirable substance in the antitoxic serum is lost by the refining process Not only we ourselves, but the resident and attending physicians watching the cases in the contagious disease hospitals noted that the rashes following the injections of the globulin solution seemed to be less severe than those which followed the injection of whole serum. It was especially noted that there were very few who had any constitutional disturbances even when the rashes did appear.

TABLE I.—Results of Injecting the Mixed Antitoxic Horse Serum in Fifty Cases of Diphtheria Occurring in Children under Ten Years of Age.

Case.		Age in years.	Antitoxin units given.		Delcterious effects. Constitutional disturbances.	Rash.
1	x	0.9	10,000	M	Marked, 5° rise of temperature.	Eighth day, general erythema lasting six days.
2		1	3,000	S	Slight	Tenth day. urticaria general.
3		1	14,000	M	High temperature, due partly to pneumonia.	Fourteentb day, urticaria lasting five days.
4	I	1.3	15,000	M	Moderate, 2° rise of temperature.	Eighth day, general erythema.
5	x	1 9	5,000	S	.....	
6	I	1.5	35,000	S	High temperature aud marked disturbance.	Sixth day, urticaria; eigbth day, general erythema of very severe type.
7	x	1.3	10,000	M	.....	
8	x	1.3	13,500	M	Moderate, with 1° rise of temperature.	Fourth day, severe general urticaria lasting three days.
9	x	1.6	10,000	M	Masked by pneumonia having 106° temperature.	Seventh day, severe general erythema.
10		1.7	10,000	M	Moderate, with 2° rise of temperature.	Sixth day, severe general erythema lasting three days.
11	x	1.9	10,000	M	Marked, with 3° rise of temperature.	Third day, morbilliform; eleventh day, severe general urticaria.
12		1.5	7,000	M	Slight.	Fourteenth day, erythema aud urticaria general for two days.
13	x	1.5	10,000	S	Slight, with 1.5° rise of temperature.	Second day, quite severe erythema lasting one day.
14	x	2	17,000	M	Slight, with 1.5° rise of temperature.	Third day, very severe urticaria for two days.
15	x	2	10,000	M	Marked, but possibly due to sepsis.	Third day, urticaria and erythema very severe lasting fifteen days.
16		2	3,500	S	.....	
17	I	2.5	7,000	M	Moderate, 2° rise of temperature.	Fifth day, severe urticaria for six days.
18	x	2.5	14,000	M	Slight.	Thirteenth day, severe urticaria for three days.
19	x	2	3,000	S	Slight.	Twelfth day, geueral urticaria for two days.
20		2	10,000	M	Slight.	Sixth day, general urticaria for three days.
21		1.5	7,000	S	.....	
22		2.5	7,000	S	Extremely severe, 3°-6° for ten days.	Eighth day, morbilliform continued and intense for ten days.
23		2	7,000	S	Extremely severe, 2°-4° for one week.	Fifteenth day, morbilliform coutinued and intense for eight days.
24	x	2.5	8,000	S	Severe, 4° rise of temperature.	Tenth day, erythema for two days; seventeenth, second lasted six days.
25	x	2.5	7,000	M	Severe, 4° rise of temperaturc.	Seventh day, erythema for two days; twelfth day, second lasted five days.
26		2.8	12,500	M	Slight.	Twenty-second day, general erythema.
27	x	3	10,000	M	Severe, but possibly due to pneumonia.	Fourteenth day, erythema for five days.
28	x	3	10,000	M	.....	
29	I	3	8,000	M	None, except 1.50 rise of temperature.	Sixth day, urticaria for two days.
30	x	3	7,500	M	.....	
31	I	3	10,000	M	Severe, 5° rise of temperature for one week.	Thirteenth day, severe erythema for one week until death.
32	x	3	3,000	S	.....	
33	x	3	14,000	S	.....	
34		3	7,500	S	Severe, for ten days 4° rise of temperature.	Thirteenth day, severe erythema lasting ten days.
35		3	14,000	M	Very severe with 4°-7° rise of temperature for ten days.	Eighth day, general erythema over whole body for ten days.
36		4	3.750	S	.. ...	
37	x	4	10,000	M	.....	Twelfth day, severe erythema.
38		4	10,000	S	.....	
39	x	4	10,000	M	Moderate, 2° rise of temperature.	Sixth day, general erythema lasting three days.
40	x	4	5,000	S	.....	Fifth day, general erythema lasting three days.

TABLE I. (Continued.)

Case.		Age in years.	Antitoxin units given.		Deleterious effects.	
					Constitutional disturbances.	Rash.
41	x	4	10,000	M	Marked, 4°-6° rise of temperature.	Tenth day, very severe, lasting five days until death.
42		4.5	12,000	S	.....	
43		5	10.000	M	Moderate, 3° rise of temperature.	Fifth day, very severe, urticaria lasting five days.
44	x	6	5.000	S	.....	
45		6	10,000	M	Moderate, 2° rise of temperature.	Sixth day, general erythema lasting three days.
46		7.5	7,500	M	Marked, 4° rise of temperature.	Fifth day, general erythema. Eighth day, general urticaria.
47		9	10,000	S	Slight.	
48	x	8	3,750	S	.....	
49	x	8	3,750	S	.....	
50	x	9	3,750	S	.....	
50		3.24 years average.	Average units per case 9,250	M 28 S = 22	Thirty-five developed constitutional disturbances.	Thirty-six developed rashes.

x = intubated. I = croup. M = marked severity (of which eighteen were intubated). S = slight severity. .... = absent.

As the serum supplied by different horses, or from the same horse at different times, is known to vary in the rashes and other after-effects it produces, and as it is, therefore, difficult accurately to compare the globulin solution and the whole serum derived from different bleedings, it was decided to make a decisive test by collecting a quantity of serum from four different horses, mixing it thoroughly, and then after precipitating one-half, to treat an equal number of patients simultaneously with the whole serum and with the globulin solution. These tests were carried out chiefly in the Willard Parker Hospital, but a few also in the Riverside Hospital. We are indebted to Drs. Lynah and Watson, the resident physicians in charge of these two hospitals, for their interest and aid.

It soon became evident that the serum we had chosen for the test was of such a character that eruptions and constitutional disturbances usually appeared in those injected with the whole serum. Whether it was because the serum from four long-treated horses had been mixed, or whether because of some other reason, it is certain that this serum produced more after-effects than any lot we had used in the hospital since 1899. These after-effects were so marked and occurred in such a large proportion of the children that we had to abandon the use of the whole serum. The rashes in those given the globulin preparation were much less severe. In persons over ten years of age almost no rashes occurred after either preparation. The patients treated with the whole serum and the antitoxic globulins were most carefully watched by us and the course of the disease, as well as the after-effects, noted.

TABLE II.—Results of Injecting Refined Antitoxin (Antitoxic Globulins) Made from Serum Obtained from the same Horses and at the same Bleedings as the Antitoxic Serum Used in the Cases Given in Table I.

No.	Age.		No. units of antitoxin. Severity.		Deleterious effects due to antitoxin. Constitutional disturbances.	Rash.
1	0.5		7,000	S	.....	Sixth day, moderate urticaria and erythema lasting four days.
2	0.9	X	15,000	M	.....	Second day, general erythema lasting two days.
3	1	I	10,000	M	Masked by pneumonia.	Ninth day, general erythema lasting five days until death.
4	1.5		5,000	S	.....	
5	1.5	X	12,000	M	Rise of 1° of temperature.	Third day, urticaria for one day.
6	0.3	X	7,000	S	.....	Second day, geueral erythema for three days
7	1.5		12,000	M	.....	
8	.4		3,000	S	.....	
9	1.2	X	10,000	M	.....	Tenth day, urticaria lasting four days
10	1.2		15,000	M	.....	Eighth day, urticaria lasting two days.
11	1.5	X	12,000	M	.....	Eighth day, urticaria, pretty severe, lasting three days.
12	1.3	X	12,000	M	.....	
13	0.9		5,000	M	.....	Fourth day, erythema lasting thirty-six hours.
14	.5		7,000	M	.....	
15	1.5	X	12,000	M	.....	Fifth day, urticaria for one day.
16	2		10,000	M	.....	
17	2	I	12,000	M	.....	
18	2	I	10,000	M	.....	Seventh day, mild urticaria for one day.
19	2	I	12,000	S	.....	
20	2		24,000	M	.....	
21	2	I	7,000	S	.....	
22	2		7,000	S	.....	
23	2	X	10,000	S	Masked by pneumonia.	Thirteenth day, general erythema lasting three days.
24	2		10,000	M	.....	Tenth day, general erythema lasting three days.
25	2.5	X	12,000	M	Rise of 1° of temperature.	Fifth day, urticaria, then erythema —together lasting five days.
26	2.5	X	12,000	M	Rise of 3° of temperature for twelve hours, then normal.	Seventh day, urticaria for two days.
27	3	X	17,000	M	.....	
28	3	X	10,000	M	.....	Eleventh day, erythema for two days.
29	3	I	12,000	M	.....	[days.
30	3	X	20,000	M	.....	Sixth day, urticaria lasting two
31	3	X	12,000	M	.....	Second day, slight general erythema lasting twenty-four hours.
32	4		7,000	S	.....	Sixth day, urticaria lasting two days.
33	4.5	X	8,000	M	.....	
34	4	I	12,000	S	.....	
35	4.5	I	12,000	M	.....	
36	4	X	12,000	M	.....	
37	4		12,000	M	Rise of 2° of temperature for one day.	Eighth day, severe urticaria, traces lasting five days.
38	4		12,000	S	.....	
39	5		5,000	S	.....	
40	5		12,000	M	.....	
41	5		3,000	S	.....	
42	5		19,000	M	Rise of 2° of temperature for one day.	Sixth day, severe erythema, traces lasting seven days.
43	5.5		3,000	S	.....	
44	6		10,000	S	.....	
45	6		3,000	S	.....	Sixth day, urticaria and erythema for three days.
46	6		4,000	S	.....	Seventh day, urticaria for two days.
47	7	X	12,000	M	.....	
48	8.5	X	24,000	M	.....	
49	9		7,000	S	.....	
50	9		3,500	S	.....	
50	3.18 years average.		10,600 units average injections.	M = 31 S = 19	Constitutional disturbances, 5; possibly 7.	Rashes In 23.

X = intubated. I = croup. M = marked severity. S = slight severity. . . . . = absent. Of the fifty there were thirty-one of marked severity; eighteen of these were intubated.

TABLE III.—Comparative Table Giving a Summary of the Constitutional and Local Reactions Obtained in the Treatment of Fifty Cases of Diphtheria in Young Children with a Lot of Antitoxic Serum Derived from Four Horses and of an Equal Number of Similar Cases Treated with a Solution of the Antitoxic Globulins Derived from a Portion of the Same Lot of Serum.

	Children who were treated with the whole serum.	Children who were treated with the antitoxic globulins.
Marked constitutional symptoms accompanied by severe and persistent rash in	28 per cent.	0 per cent.
Moderate constitutional symptoms accompanied by a well-developed erythema or urticaria	18 "	4 "
Very slight constitutional disturbance accompanied by a more or less general rash	20 "	"
No appreciable constitutional disturbance, but a more or less general urticaria or erythema.	4 "	34 "
No appreciable after-effects whatever	30 "	54 "

TABLE IV.—Duration of Rashes.

	Days.								
	1	2	3	4	5	6	7	8 and over.	Total rashes.
Antitoxic globulin cases	4	7	5	3	3		2	...	23
Whole serum cases	1	4	10	1	10	3	2	5	36

After all the tested patients had become fully convalescent or had left the hospital, the histories were finally gone over and compared. It was found that fifty children under ten years of age treated with the whole serum had lived at least nine days, or long enough for the development of serum effects. The first fifty consecutive cases in children under ten years treated with the antitoxic globulins precipitated from the same lot of serum and living nine days or over were taken to compare with these. Table I gives the salient points for each case treated with the whole serum and Table II the same for those treated with the solution of antitoxic globulins. Tables III and IV summarize these points.

It is noticeable that not only were the rashes more frequent, but also much more persistent in the patients who received the whole serum. Twenty-three rashes following the use of the whole serum lasted over three days in this series, as against only six in the antitoxic globulin cases.

SUMMARY AND CONCLUSIONS. The results obtained in these series of one hundred cases are so definite that it seems safe to conclude that the removal of a considerable portion of the non-antitoxic globulins, as well as the albumins from the serum by the Gibson method, has eliminated much of the deleterious matter from the serum, so that severe rashes, joint complications, fever, and other constitutional disturbances are less likely to occur from the antitoxic

globulins than from the antitoxic serum from which it was obtained. The globulin preparation when tested by animal experiments appears to retain all the antitoxic properties of the whole serum. The portion of the globulins still accompanying the antitoxin in the Gibson preparation is shown to be capable of exciting rashes and occasionally constitutional disturbances, although, as stated above, to a less extent than the serum. It is almost certain that methods will be devised to refine antitoxin still farther, and so possibly eliminate all appreciable deleterious effects of the antitoxic serum.

Whether this globulin solution will be much less likely than the serum to cause collapse in the rare cases of peculiar susceptibility, such as in a certain percentage of those suffering from status lymphaticus, is still undetermined. It has now been used in several thousand cases of diphtheria without accident.

The concentration of antitoxin made possible by the elimination of the non-antitoxic substances is not only a convenience, but of distinct clinical importance, as it tends to encourage large doses.

The antitoxic globulin solution, like the serum, tends to become slightly cloudy when kept at moderate or high temperature, and substances such as solutions of carbolic acid and trikresol are especially likely to cause a precipitate to develop. The antitoxin in the globulin preparation retains its potency about as long as that in the whole serum.